Lrit3 Cas9-CKO Strategy

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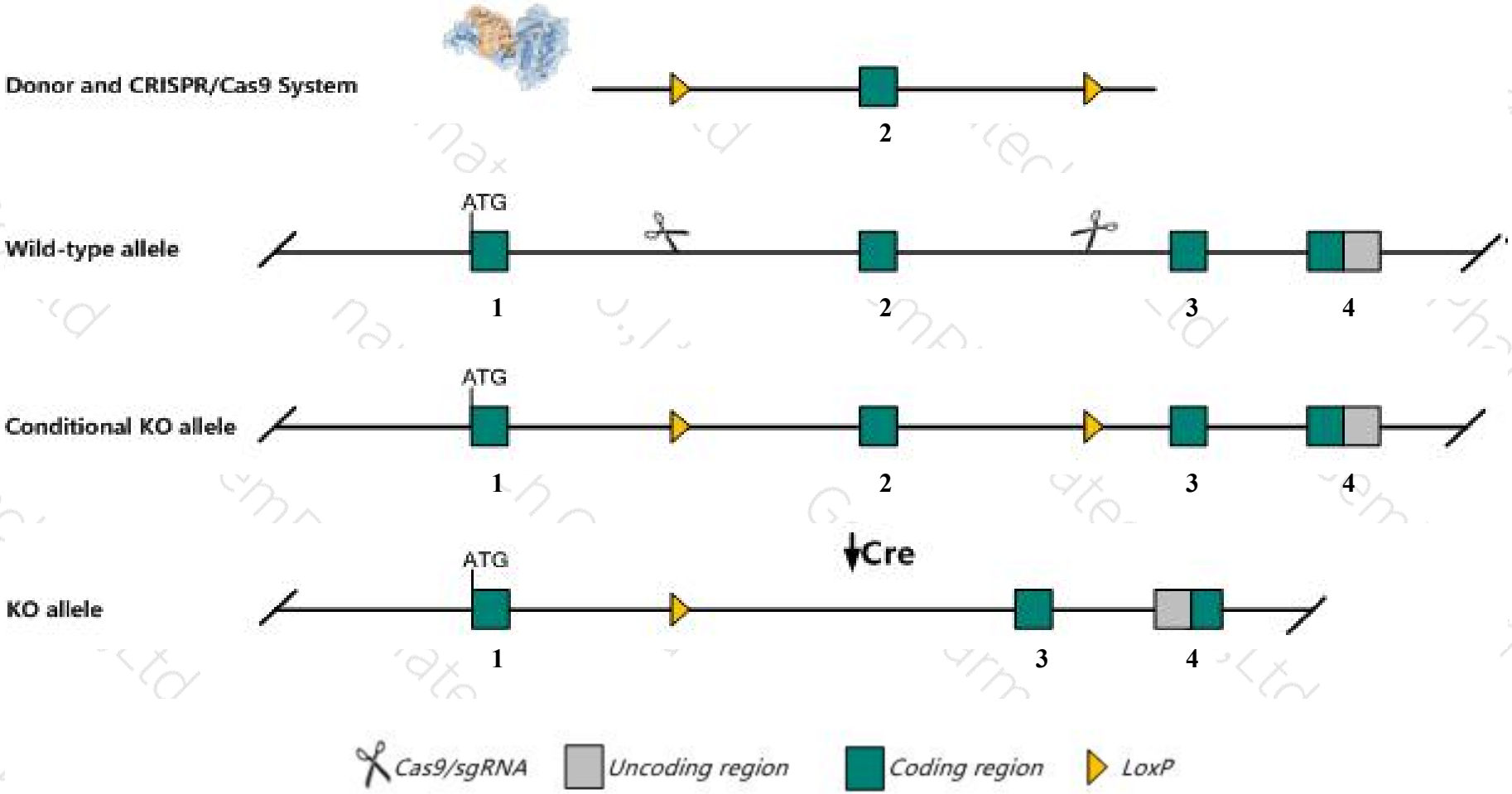
Project Overview

Project Name	***Lrit3***
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Lrit3* gene. The schematic diagram is as follows:



Technical routes

➢ The *Lrit3* gene has 3 transcripts. According to the structure of *Lrit3* gene, exon2 of *Lrit3-202*(ENSMUST00000185462.6) transcript is recommended as the knockout region.The region contains 473bp coding sequence. Knock out the region will result in disruption of protein function.

➢ In this project we use CRISPR/Cas9 technology to modify *Lrit3* gene. The brief process is as follows:sgRNA was transcribed in vitro, donor vector was constructed.Cas9, sgRNA and Donor were microinjected into the fertilized eggs of C57BL/6JGpt mice.Fertilized eggs were transplanted to obtain positive F0 mice which were confirmed by PCR and sequencing. A stable F1 generation mouse model was obtained by mating positive F0 generation mice with C57BL/6JGpt mice.

➢ The flox mice was knocked out after mating with mice expressing Cre recombinase, resulting in the loss of function of the target gene in specific tissues and cell types.

Notice

➢ According to the existing MGI data,mice homozygous for a targeted allele show a selective absence of the ERG b-wave with a normal a-wave component under scotopic conditions, as well as variable ERG responses with larger a-wave amplitudes, shorter b-wave amplitudes, and longer implicit times of both waves under photopic conditions.

➢ The *Lrit3* gene is located on the Chr3. If the knockout mice are crossed with other mice strains to obtain double gene positive homozygous mouse offspring, please avoid the two genes on the same chromosome.

➢ This strategy is designed based on genetic information in existing databases.Due to the complexity of biological processes,all risk of loxp insertion on gene transcription, RNA splicing and protein translation cannot be predicted at existing technological level.

Gene information （NCBI）

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Lrit3 leucine-rich repeat, immunoglobulin-like and transmembrane domains 3 [Mus musculus (house mouse)]

Gene ID: 242235, updated on 13-Mar-2020

Summary

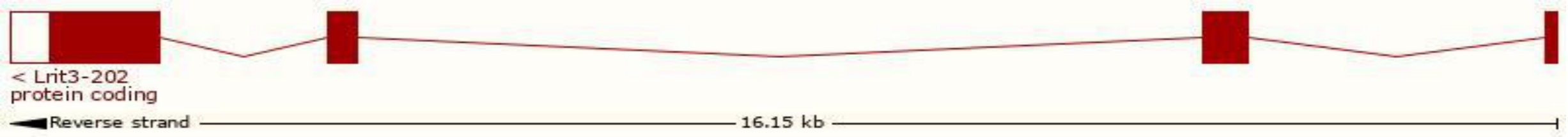
Official Symbol	Lrit3 provided by MGI
Official Full Name	leucine-rich repeat, immunoglobulin-like and transmembrane domains 3 provided byMGI
Primary source	MGI:MGI:2685267
See related	Ensembl:ENSMUSG00000093865
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	Gm421
Expression	Low expression observed in reference datasetSee more
Orthologs	human all

Transcript information（Ensembl）

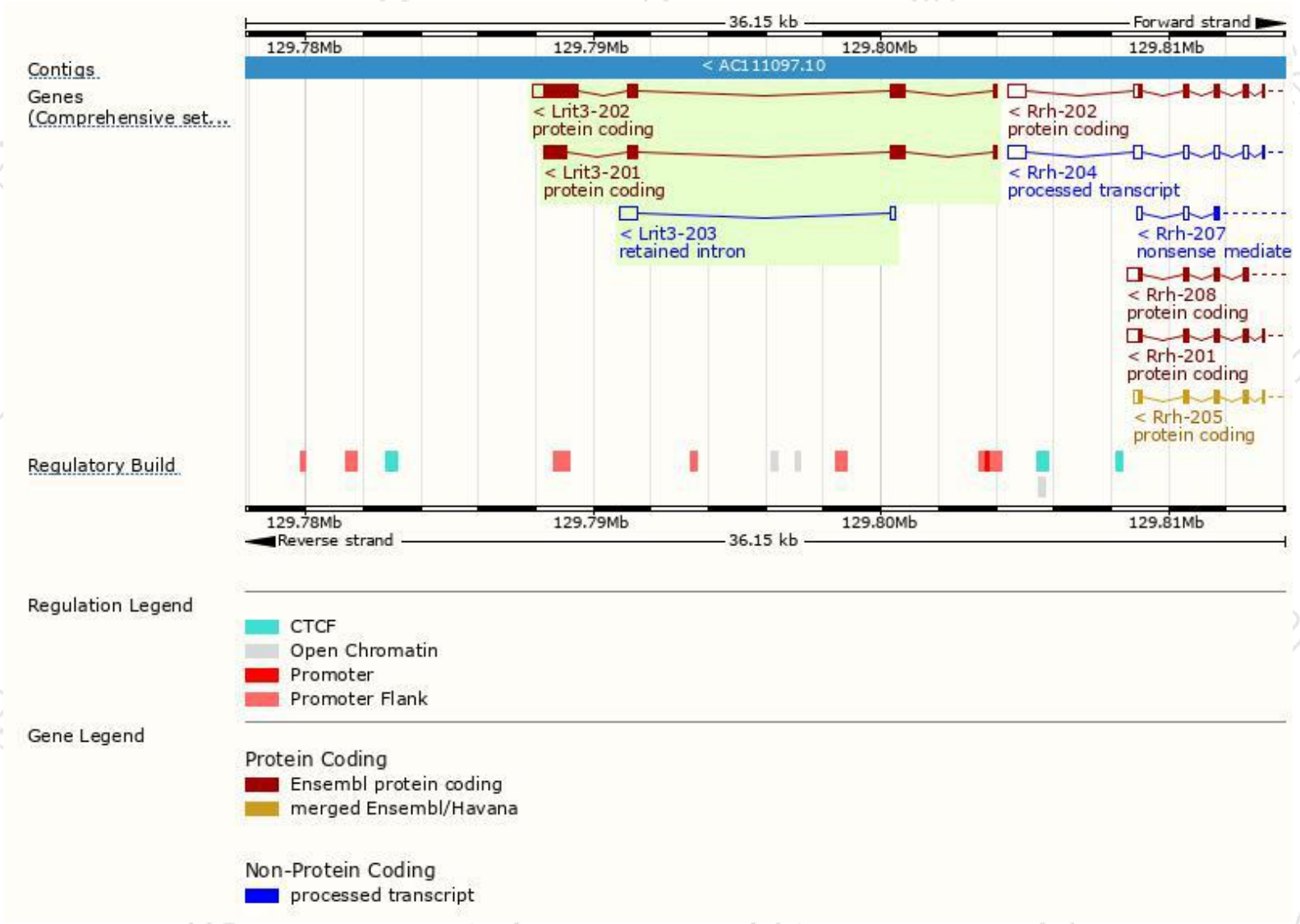
The gene has 3 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Lrit3-202	ENSMUST00000185462.6	2456	681aa	Protein coding	CCDS57256	W8DXL4	TSL:1 GENCODE basic APPRIS P2
Lrit3-201	ENSMUST00000179187.1	1683	560aa	Protein coding	-	J3QNQ8	TSL:5 GENCODE basic APPRIS ALT2
Lrit3-203	ENSMUST00000188978.1	773	No protein	Retained intron	-	-	TSL:3

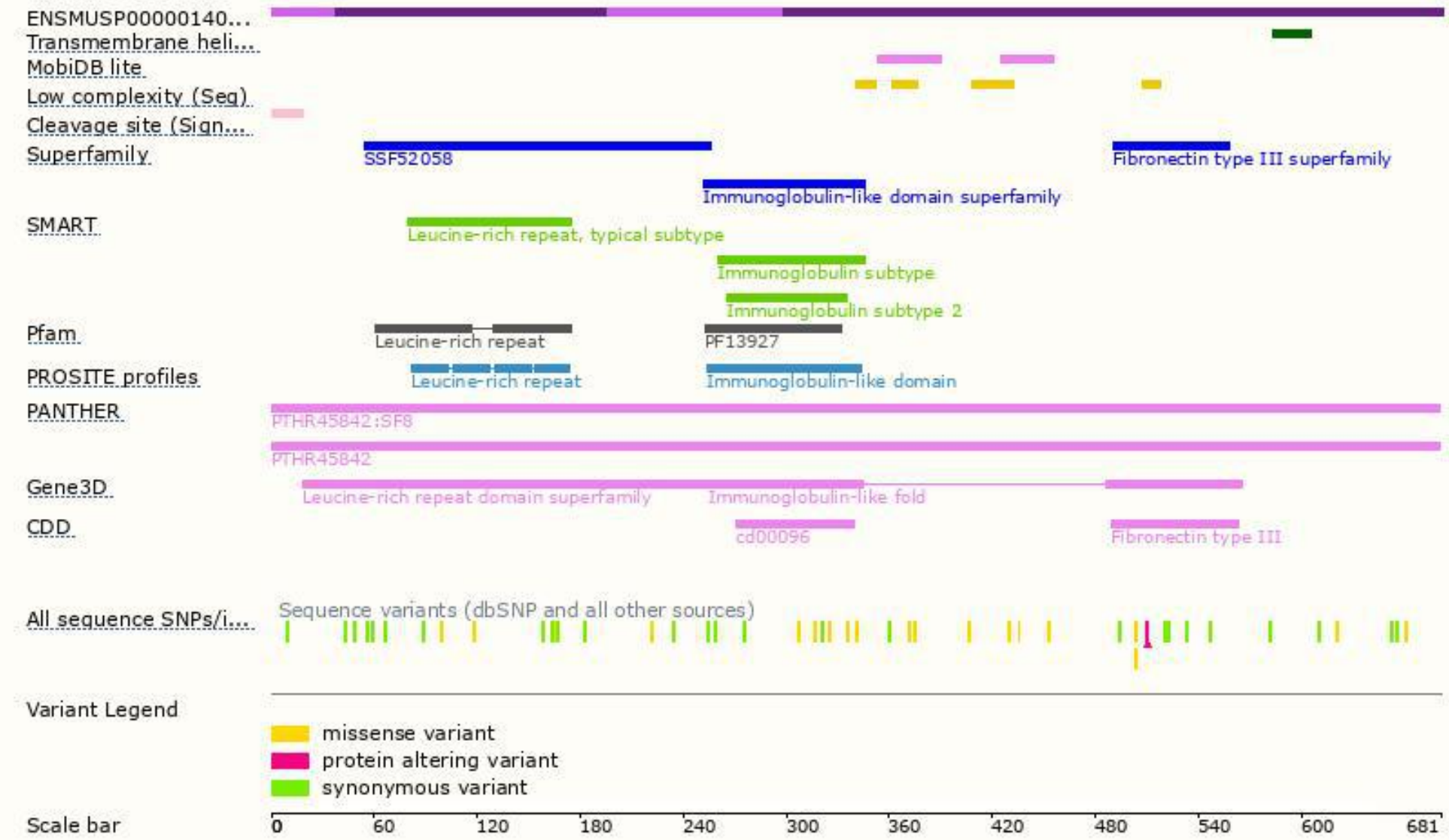
The strategy is based on the design of *Lrit3-202* transcript,the transcription is shown below:



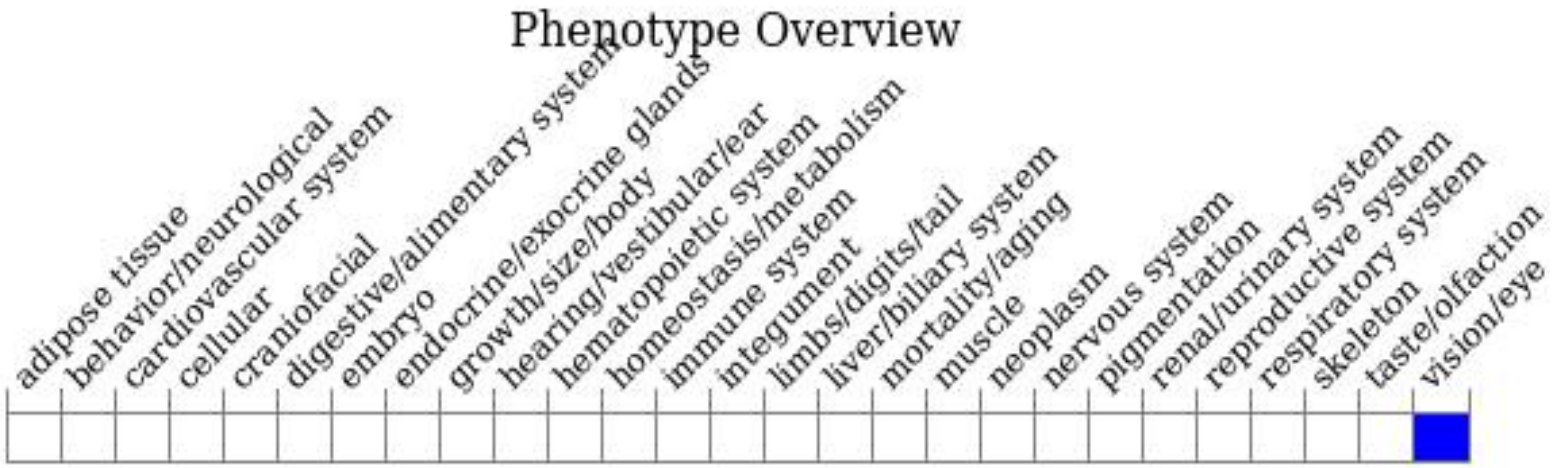
Genomic location distribution



Protein domain



Mouse phenotype description(MGI)



Phenotypes affected by the gene are marked in blue.Data quoted from MGI database(http://www.informatics.jax.org/).

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If you have any questions, you are welcome to inquire.

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